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Matching Rodents to People: A Humanized Mouse Model of iAs Methylation

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Environmental exposure to inorganic arsenic (iAs) confers an increased risk of cancer, type 2 diabetes, and cardiovascular, respiratory, and neurological disorders.[1] The underlying mechanisms have been difficult to study in animal models because mice metabolize iAs much more quickly than humans.[2,3] A team of researchers, led by Miroslav Stýblo, Beverly Koller, and John Snouwaert at the University of North Carolina at Chapel Hill, described in *Environmental Health Perspectives* the generation of mice with a more human-like iAs metabolism.[4]

iAs is metabolized by the enzyme coded by the *AS3MT* gene, which is located adjacent to BLOC-1-related complex subunit 7 (*BORCS7*). These two genes are believed to share regulatory elements.[5] The AS3MT enzyme adds methyl groups to iAs to produce monomethyl-As (MAs) followed by conversion to dimethyl-As (DMAs).[6] In mice, this detoxification step results in the urinary excretion of almost all iAs as DMAs. The less efficient human metabolism does not complete the conversion process; instead, it produces the intermediate MAs, which is excreted in the urine along with iAs and DMAs.[3]

In the new study, the researchers inserted the human genes *AS3MT* and *BORCS7* into the orthologous (equivalent) mouse *Borcs7/As3mt* locus, a process known as syntenic replacement. This "humanization" by syntenic replacement generated a model system with greatly improved translational potential, according to the authors.

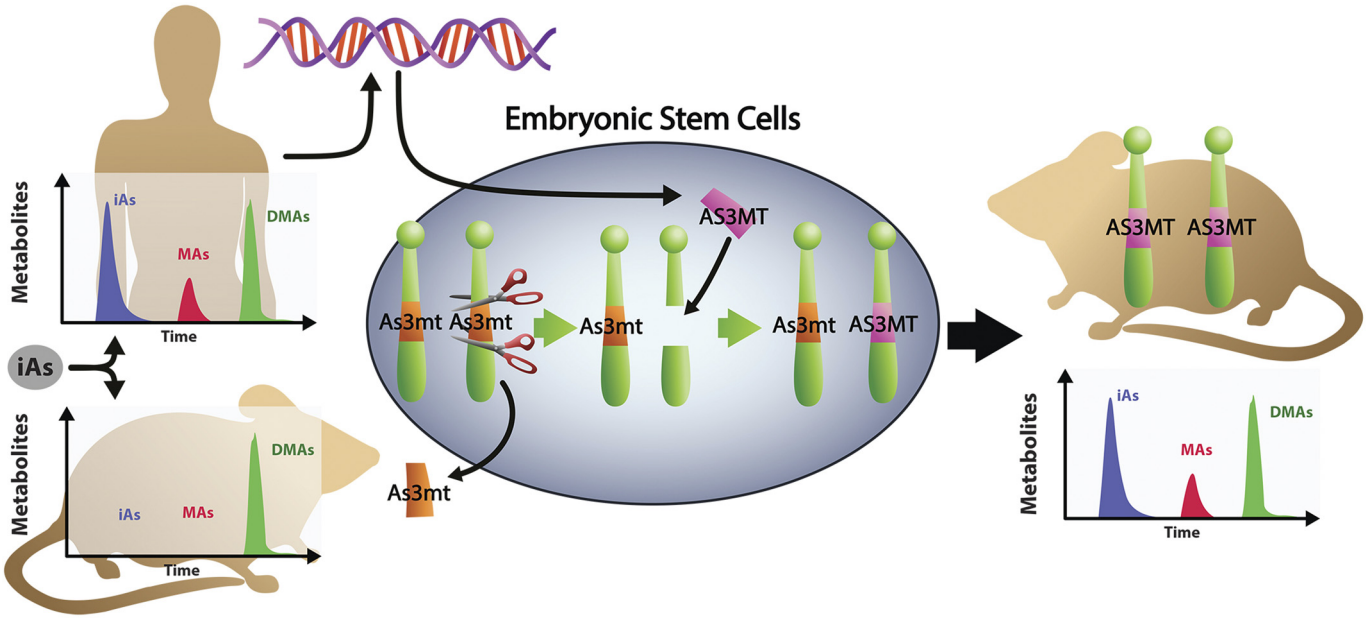
"In my career of more than thirty years, this was the first time our working hypothesis materialized almost completely in the results," says Stýblo, a professor of nutrition and the paper's senior author. "The distribution of urinary iAs metabolites in the humanized mice was an almost perfect match with human urine."

In the new study, mice consumed 400 μg/L iAs in drinking water for 4 weeks, a level far higher than typical human exposures. (The World Health Organization drinking water standard is 10 μg/L,[7] although an estimated 5 million Bangladeshis regularly consume drinking water with concentrations above 200 μg/L.[8]) Mice with two copies of the wild-type allele at *Borcs7/As3mt* (WT/WT) excreted more than 98% of total arsenic as DMAs in the urine, with undetectable levels of MAs. In mice with two copies of the human allele (Hs/Hs), however, the range was 7–11% for urinary MAs, 38% for iAs, and 52–56% for DMAs. This closely resembles the profile of arsenic metabolites in human urine.[9] Total arsenic levels in the livers and kidneys of Hs/Hs mice were also similar to predicted levels for the respective human tissues and substantially higher than in WT/WT mice.

Humanization by syntenic replacement has advantages over traditional transgenic models, which involve deleting an animal gene and inserting the human ortholog elsewhere in the genome.[10] For example, says first author Koller, the new model is ideal for studies of *AS3MT* mutations that result in slower or faster methylation of iAs, of interactions between *AS3MT* and genes in the same metabolic pathway, and of gene–environment interactions.

"In a transgenic animal, the human transgene differs from the original in copy number and regulatory elements since it's located in a different part of the genome," explains Koller, an associate professor of genetics. "That makes it more difficult to isolate the effect of new experimental factors, compared to mice that were humanized by syntenic replacement."

Another advantage is the ability to compare the expression of mouse and human genes in one heterozygous Hs/WT animal.



In both humans and mice, iAs is converted to mono- and dimethylated metabolites in reactions catalyzed by arsenic methyltransferase. However, this conversion is much more efficient in mice than in humans—so efficient, in fact, that ingested iAs is barely detectable in mouse urine. For the new study, investigators replaced the mouse arsenic methyltransferase gene (*As3mt*) with its human ortholog (*AS3MT*) to produce a "humanized" mouse that metabolizes iAs in much the same way as humans. Note: DMAs, dimethyl-arsenic; iAs, inorganic arsenic; MAs, monomethyl-arsenic. Image: Joseph Snouwaert.

"Remarkably, we observed in mice the same elevated adrenal gland expression of the human gene that's been reported for humans," says Koller. This suggests that much of the human *AS3MT* regulatory region has also been transferred to the mouse genome.

Beyond the improved ability to translate iAs toxicological findings from mice to humans, the new model may help in understanding if and how *AS3MT* or nearby genes influence behavior. Neural tissue of Hs/Hs mice expressed a human-specific *AS3MT* splicing variant ($AS3MT^{d2d3}$), which is reported to confer an increased risk of schizophrenia.[11]

The researchers examined iAs metabolites in both urine and feces. "The fecal excretion of humanized mice seemed to compensate somewhat for their slower urinary excretion," notes Stýblo. This finding was of particular interest to Seth Walk, an associate professor of microbiology and immunology at Montana State University, who was not involved in the study.

"We now know from gut microbiome studies in our lab[12] and others[13,14] that fecal excretion [in mice] strongly influences iAs toxicity—meaning that microbial dysfunction increases the risk of iAs-associated diseases," says Walk. "Studying both urine and feces[15,16] provides a much better understanding of total body burden and will likely become the new standard."

Walk calls the study a "significant step forward in the field," as does Mary Gamble, an associate professor of environmental health sciences at Columbia University, who also was not involved in the work. "Translating studies of arsenic in rodents to humans has been hampered by the substantial species differences in arsenic methylation capacity," says Gamble. "It will be very exciting to see how these humanized mice respond to longer-term exposure with regard to health outcomes."

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